

CARBONYL COMPOUNDS ASSOCIATED WITH THE OFF-FLAVOR IN SPONTANEOUSLY OXIDIZED MILK

SUMMARY

Studies on the carbonyl compounds in the butteroil of spontaneously oxidized whole milk indicate that the mechanism involved is typical of classic lipid autoxidation. The C_5 through C_{18} saturated aldehydes, C_6 to C_{11} alk-2-enals, and C_8 to C_{12} alk-2,4-dienals were tentatively identified in this product as their 2,4-dinitrophenylhydrazones, on the basis of their behavior on magnesia, ultraviolet spectra, column partition chromatographic retention volumes, paper chromatographic properties, or gas chromatographic characteristics of the regenerated compounds. Flavor studies in conjunction with quantitative carbonyl analyses of 14 samples of butteroil from nonoxidized and spontaneously oxidized milks indicate that the alk-2,4-dienals, especially 2,4-decadienal, play a significant role in the off-flavor of this product, whereas the saturated aldehydes are of little significance in this respect. It is suggested that the C_{11} to C_{18} saturated aldehydes are present in fluid milk not as products of autoxidation, but rather as the result of either milk lipid synthesis or hydrolysis of plasmalogens or neutral plasmalogens during pasteurization.

It is generally accepted that carbonyl compounds play a major role in the flavor of oxidized dairy products. Qualitative analyses of oxidized products (butteroil, cream, dry milk) have indicated that a number of carbonyls are important to the over-all flavor of the products (2, 3, 13). The literature, however, is lacking with respect to the compounds liberated in fluid milk which has undergone spontaneous oxidation. Because of the practical importance and confusing nature of this phenomenon, it was deemed advisable to study the carbonyls in the fat phase of this product and to compare the results with those reported for other dairy products.

EXPERIMENTAL PROCEDURE

Milk used in this study was obtained from individual cows of the Beltsville herd with histories of producing milk susceptible to spontaneous oxidation. The milk from two to four successive milkings, held at 40 F, was heated at 150 F for 6 min, cooled, and stored at 35 F in the dark. After storage, the milk was warmed to 75 F and separated with a DeLaval¹ laboratory separator. The cream fraction was cooled to 50 F and churned into butter in a Hamilton Beach¹ Model 30 Drink Mixer. But-

teroil was obtained by centrifuging the butter heated to 90 F on a steam bath with constant stirring.

Flavor studies. Fluid milk samples were submitted to an experienced taste panel for total flavor evaluation just before the start of analysis. Reconstituted samples were prepared by mixing 16 ml of butteroil into 400 ml of fresh skim milk in a Waring Blender.¹ Reconstituted samples were held overnight at 40 F before submitting to the taste panel. The interval between flavor evaluation of the fluid milk and its corresponding reconstituted sample did not differ by more than 24 hr.

*Saturated aldehyde 2,4-dinitrophenylhydrazones.*² The methods for the isolation and characterization of the saturated aldehydes are similar to those previously reported (12). Two hundred milliliters of butteroil were made up to one liter with carbonyl-free hexane (17), filtered through glass wool, and passed through a chromatographic tube containing 30 g of Analytical Grade Celite impregnated with 18 ml of a saturated solution of sodium bisulphite. Following passage of the solution, the packing was washed free of fat with purified hexane and the aldehydes recovered by decomposing the bisulphite with 200 ml of a 20% aqueous potassium carbonate solution. The free carbonyls were recovered by extracting the solution and packing with three 100-ml portions

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¹The use of trade names is for the purpose of identification only, and does not imply endorsement of the product or its manufacturer by the U. S. Department of Agriculture.

²Hereafter abbreviated as DNPhydrazones.

of carbonyl-free hexane. The carbonyl-hexane solution was dried with sodium sulphate, filtered, and the DNPhydrazones prepared on a reaction column according to the procedure of Schwartz et al. (16). The saturated aldehyde DNPhydrazones were eluted, as a class, from adsorptive magnesia with an increasing concentration of methanol in chloroform (18).

Unsaturated aldehyde DNPhydrazones. Buterol (500 ml) was made up to two liters with carbonyl-free hexane, filtered through glass wool, and divided into four 500-ml samples. Each sample was passed through a DNPhydrazine reaction column (16) and the alk-2-enal and alk-2,4-dienal fractions isolated from other DNPhydrazone classes according to the procedure of Schwartz et al. (18).

Identification of DNPhydrazones. The individual carbonyl classes were each separated by column partition chromatography employing columns of 30 g Celite as support and the acetonitrile-hexane solvent system of Corbin et al. (1). The elution of the saturated aldehyde DNPhydrazones was monitored by a continuous flow analyzer (Canal Industrial Corp.,

Bethesda, Maryland¹) using a 340 m μ filter. The alk-2-enal and alk-2,4-dienal eluates were collected fractionally (5-ml fractions) and read in a Beckman Model B Spectrophotometer¹ at the appropriate wavelengths (1). Evidence for identification of the DNPhydrazones in each class was obtained by comparing the retention volumes of the unknown DNPhydrazones in the column partition system with those of known compounds. Additional evidence as to the identities of the compounds was obtained by ultraviolet studies in hexane and paper chromatography by the methods of Huelin (8) and Klein and deJong (9). Only two compounds, hexanal and nonanal, were obtained in sufficient quantity for confirmation by mixed melting point. The long-chain saturated aldehydes were further identified by regenerating the DNPhydrazones with levulinic acid, followed by gas chromatography under the conditions previously reported (12).

Quantitative carbonyl analysis. Optical density determinations of the DNPhydrazones were obtained in a definite volume of hexane at the wavelengths of maximum absorption. Concen-

TABLE 1
Aldehydes tentatively identified in the fat phase of spontaneously oxidized whole milk

Suspected DNPhydrazone ^a	Adsorption maximum in hexane (m μ)		Paper chromatography Rf values	
	Observed	Reported	Unknown	Authentic
Pentanal	337	335	0.67 ^c	0.67
Hexanal ^e	335	335	0.74 ^c	0.75
Heptanal	335	335	0.84 ^c	0.84
Octanal	335	335	0.72 ^d	0.72
Nonanal ^e	335	335	0.68 ^d	0.68
Decanal	335	335	0.66 ^d	0.66
Undecanal	335	335	0.58 ^d	0.58
Dodecanal	335	335	0.53 ^d	0.54
Tridecanal ^b	335	335	0.51 ^d	0.51
Tetradecanal ^b	335	335	0.49 ^d	0.48
Pentadecanal ^b	335	335	0.46 ^d	0.45
Hexadecanal ^b	335	335	0.42 ^d	0.42
2-Hexenal	353	355	0.67 ^c	0.66
2-Heptenal	353	355	0.73 ^c	0.71
2-Octenal	353	355	0.76 ^c	0.75
2-Nonenal	353	355	0.86 ^c	0.84
2-Decenal	353	355	0.72 ^d	0.71
2-Undecenal	353	355	0.64 ^d	0.65
2,4-Octadienal	368	370	0.71 ^c	0.71
2,4-Nonadienal	368	370	0.75 ^c	0.75
2,4-Decadienal	370	370	0.80 ^c	0.80
2,4-Undecadienal	370	370	0.84 ^c	0.85
2,4-Dodecadienal	370	370	0.69 ^d	0.70

^a Tentative identifications based on class separation on adsorptive magnesia and column partition chromatography retention volumes.

^b Gas chromatography revealed both normal and branched-chain components.

^c Method of Huelin.

^d Method of Klein and deJong.

^e Conclusive identification by mixed melting point determinations.

trations in parts per billion (ppb) of 4% milk were obtained by calculation.

RESULTS AND DISCUSSION

The quantitative isolation of flavor compounds from fluid milk is a difficult problem. Vacuum distillation techniques, which have been applied (2, 20) to such products, are limited in their ability to recover the higher boiling compounds. The methods used in this study have the advantage of recovering these compounds to an extent which makes their tentative identification possible despite the extremely small quantities present. However, our approach has certain limitations. The recovery of butteroil from fluid milk does not represent an absolute procedure for the recovery of all flavor compounds in this product. The nature and quantity of the compounds accompanying the butteroil are dependent on the partition coefficients of the compounds between the skim-milk and butteroil fractions of the product. This was readily observed during the isolation of the butteroil fraction of oxidized milk. The residual skim-milk had an oxidized flavor. However, the product resulting from the reconstitution of the butteroil fraction into fresh skim-milk also exhibited the typical oxidized flavor. Hence, despite the limitations of the method employed, the compounds associated with the butteroil fraction of milk appear to be representative of those which can impart a typical oxidized flavor to milk.

The conclusive identification of the carbonyls in the butteroil fraction of spontaneously oxi-

dized milk by melting point studies, with the exception of nonanal and hexanal, was not possible, due to the extremely small quantities present in the products. (A laboratory accident prevented the conclusive identification of Heptanal DNPHHydrazone.) However, based on their behavior in the class separation on magnesia, ultraviolet spectra, column partition chromatographic retention volumes, paper chromatographic properties, and gas chromatographic characteristics, highly confident tentative identities were assigned. For the most part, the compounds listed in Table 1 are not unlike those reported to occur in other oxidized dairy products (4, 5, 6) and as such are representative of the by-products of classic lipid autoxidation. The failure to identify formaldehyde, acetaldehyde, propionaldehyde, 2-pentenal, 2,4-hexadienal, and 2,4-heptadienal, compounds identified by others in oxidized milk fat systems, may be attributed to the solubility characteristics of these compounds and the minute concentrations originally present in the fluid product. One can conclude, however, that these compounds are not necessary for the typical oxidized flavor of fluid milk, since the butteroil homogenized into fresh skim-milk exhibited the characteristic off-flavor in their absence.

Several carbonyls identified in this study and absent in reports of previous investigations on oxidized dairy products can be attributed for the most part to the procedures employed in this study. The C_{11} to C_{16} saturated aldehydes are known to occur in milk bound to glycerol

TABLE 2
Efficiency of bisulphite technique for extracting carbonyls from hexane and hexane butteroil solutions

Carbonyl	Fat in hexane	Carbonyl	Carbonyl extracted by bisulphite	Extracted carbonyl recovered from bisulphite
	(%)	(μ M)	(%)	(%)
Heptanal	0	6.8	100.0	95.1
	20	10.2	100.5	100.3
Decanal	0	9.5	99.2	97.3
	11	10.3	100.0	96.6
Octadecanal	0	6.2	98.1	87.3
	18	5.4	95.1	89.6
2-Tetradecenal	0	7.9	89.6	15.9 ^a
2,4-Dodecadienal	0	5.9	100.2	0.7
	19	5.2	98.4	9.3 ^a
Butanone-2	0	12.1	74.3	72.8
Undecanone-2	0	17.1	0	0
	17	18.4	0	0
Heptadecanone-2	0	11.2	3.04	71.0

^a Analyses revealed saturated aldehyde and methyl ketone contaminants present.

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in the plasmalogen (19) and butteroil (12) fractions. It seems reasonable to assume that these compounds occur in fluid milk not as products of autoxidation but as products of either milk lipid synthesis or hydrolysis during pasteurization. The latter assumption is substantiated by the quantitative results in Table 3. Despite differences in the samples analyzed, the concentration of long-chain saturated aldehydes in the milk of individual cows was not influenced by the autoxidative reaction.

The indicated presence of 2,4-dodecadialenal is, so far as is known, the first report of this compound in an oxidized dairy product. Gaddis et al. (6), however, reported its presence in oxidized animal fats and highly oxidized methyl linoleate. Its formation from the latter can not be explained on the basis of current theories of oxidation. Some of the unusual carbonyl compounds found in oxidized milk may originate from classical autoxidation of the unique fatty acids in this product. Herb et al. (7) have recently found trace quantities of a wide

variety of unique unsaturated fatty acids in milk fat.

Methyl ketones were also observed in the carbonyl analysis of both fresh and autoxidized whole milk, but are not included in Table 1. Their presence in dairy products has been shown to be dependent on heat treatment (15) and as such are of little significance in studies relating to autoxidation.

The identification of flavor compounds to be of significance must be correlated with quantitative studies. Procedures developed in this laboratory and used here are quantitative for the monocarbonyls in the isolated fat phase. However, the methods give rise to those aldehydes which are bound and acid-labile (12). Therefore, the saturated aldehydes present as a result of the autoxidative mechanism must be determined by a supplemental technique such as the bisulphite method described. As the results on known carbonyl solutions (recovered from the semicarbazone or bisulphite addition product) in Table 2 indicate, the method is quantitative so far as the saturated

TABLE 4
Carbonyl content and oxidized flavor criticisms of reconstituted milks prepared from butteroil of nonoxidized and spontaneously oxidized milks and fresh skim milk

Carbonyl	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
Concentration in parts per billion of 4% reconstituted milk						
Pentanal	1.72	0.55	1.34	0.54	0.45	0.36
Hexanal	4.48	3.78	14.96	4.49	4.99	2.99
Heptanal	3.74	2.03	6.68	2.04	2.23	1.36
Octanal	1.08	1.13	1.87	0.92	0.62	0.61
Nonanal	2.04	2.33	3.64	2.91	1.21	1.94
Decanal	0.97	1.03	1.47	1.06	0.49	0.71
Undecanal	0.34	0.41	0.41	0.24	0.14	0.16
Dodecanal	0.88	0.66	1.10	0.48	0.37	0.32
C ₁₃ -C ₁₈ sat. ald.	8.09	9.81	17.49	6.08	5.83	4.05
2-Hexenal ^a	0.18	0.06
2-Heptenal	Trace	0.74	0.20	0.25	0.13
2-Octenal	0.15	Trace	0.97	0.29	0.32	0.19
2-Nonenal	0.25	0.17	1.12	0.36	0.37	0.24
2-Decenal	0.36	0.23	2.34	0.77	0.78	0.51
2-Undecenal	0.18	0.20	2.45	0.98	0.82	0.65
2,4-Octadienal	0.19	0.06
2,4-Nonadienal	0.60	0.28	0.20	0.18
2,4-Decadienal	0.09	1.12	0.48	0.37	0.32
2,4-Undecadienal	} 0.42	} 0.32	} 0.12	} 0.21
2,4-Dodecadialenal	0.14				
Oxidized flavor	0(+)-10(-)	0(+)-10(-)	8(+)-2(-)	6(+)-4(-)	5(+)-5(-)	4(+)-6(-)

Sample A—Butteroil from 22 hour milk of Cow No. 2.

B—Butteroil from six day milk of Cow No. 2.

C—Butteroil from four day milk of Cow No. 3.

D—Butteroil from seven day milk of Cow No. 3. Reconstituted Product diluted 1:1 with fresh whole milk.

E—Butteroil from four day milk of Cow No. 3. Reconstituted Product diluted 1:2 with fresh whole milk.

F—Butteroil from seven day milk of Cow No. 3. Reconstituted Product diluted 1:2 with fresh whole milk.

^a Not detected.

aldehydes are concerned. The unsaturated aldehydes, although extracted by the bisulphite, are not recovered as a result of the irreversible addition of sodium bisulphite to the double bonds. Furthermore, the lower molecular weight methyl ketones react with sodium bisulphite to a limited extent, whereas the higher molecular weight compounds do not react at all. Hence, the direct reaction of butteroil with DNPhydrazine for isolating the unsaturated aldehydes in conjunction with the bisulphite technique for saturated compounds meet the need for quantitative recovery of the carbonyls in autoxidizing lipid systems.

Table 3 presents flavor studies in addition to quantitative carbonyl analyses of butteroil from spontaneously oxidized and nonoxidized fluid milk samples stored at 35 F for varying lengths of time (flavor criticisms other than oxidized are reported as negative for this defect). Based on published flavor threshold studies (10, 11, 14), the data in Table 3 suggest that the alk-2,4-dienal fraction, especially 2,4-decadienal, is the major contributor to the off-flavor of this product, whereas the saturated aldehydes are not present in sufficient quantities to materially affect the flavor. This observation is further substantiated in Table 4, which shows that despite having concentrations below those levels in a nonoxidized fluid milk, an off-flavor persisted in the reconstituted samples diluted to various degrees with fluid fresh milk. At the same time, the diluted samples contained levels of 2,4-decadienal (0.32-0.48 ppb) near the reported flavor threshold of 0.5 ppb for this compound (14).

Despite its implication in the off-flavors associated with the copper-induced oxidized flavor in skimmilk (4, 5), and the similarity in flavors, the contribution of the alk-2-enals to the oxidized flavor in spontaneously oxidized whole milk is debatable. The concentrations of these compounds reported in Table 3 are, for the most part, substantially below those levels reported to be detectable in milk (11).

This work indicates that the flavor compounds in spontaneously oxidized milk arise from classic lipid autoxidation, and that alk-2,4-dienals are the important flavor contributors.

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